Study code: 3000-4205
(Old code 1903005)

Study title:

Effects on gastrointestinal transit in mice. Hydroxymatairesinol.

CONFIDENTIAL

CONFIDENTIAL

Study Report

EFFECTS ON GASTROINTESTINAL TRANSIT IN MICE

HYDROXYMATAIRESINOL

Study number: **P11.14-1999**

Date: 20.8.2002 (version 2)

Sponsor:

Hormos Medical Ltd.
Tykistökatu 6A
FIN-20520 Turku
FINLAND

Sponsor Study number: 1903005

CONFIDENTIAL

PreFa
Preclinical Pharmacology Research Unit
University of Turku

Key Words: Hydroxymatairesinol (HMR), safety pharmacology, gastrointestinal transit/propulsion

1. GENERAL

1.1. SIGNATURES

Title Effects on gastrointestinal transit in mice; Hydroxymatairesinol

PreFa study number: P11.14-1999

Sponsor study number: 1903005

Testi item: Hydroxymatairesinol (HMR)

This Report version 2 replaces the 1st version dated 14.6.2000. Following changes have been made:

1. **Summary:** The route of administration of the test item has been corrected (earlier: p.o.)
2. **Section 2.3.4. Rationale for dose selection:**
   a. Reference to a study demonstrating the antitumor activity of HMR has been added.
   b. The route of administration of the test item has been corrected (earlier: p.o.)

This report is a complete and accurate account of the methods employed and the data obtained

20.8.2002

Aapo Honkanen
Study Director

date

1.2. TABLE CONTENTS

1.3. PURPOSE OF THE STUDY

The purpose of this study was to evaluate safety of the compound Hydroxymatairesinol (HMR) by assessing its effect on gastrointestinal transit in mice. In addition to HMR, the effects of another compound, HTS-101 was tested in the same experiment. Same control group (vehicle treatment) and reference compound-treated group were used in the evaluation of these compounds. The results from HMR and HTS are reported separately.

1.4. SUMMARY

NMRI mice withdrawn from food but not water for 18 h were given different doses of HMR (10, 30 or 100 mg/kg, s.c.) or vehicle (PEG 300) 90 min or reference compound medetomidine 30 min before the administration of charcoal meal (0.25 ml of 5 % charcoal in 0.8 % methylcellulose). The mice were sacrificed 15 min following the charcoal meal with CO_2 and the entire intestine was immediately removed. The distance the meal had travelled through the intestine was measured. Gastrointestinal transit was calculated as the percentage of distance travelled by the charcoal, relative to the total length of the small intestine Only medetomidine significantly inhibited the transit of charcoal meal (vehicle group 42 ± 6 % vs medetomidine group 16 ± 7 %, Mean ± S.D.) all doses of HTS-101 being without effect (10 mg/kg: 43 ± 8%; 30 mg/kg: 43 ± 8% and 100 mg/kg: 39 ± 7%). These results indicate that Hydroxymatairesinol does not alter gastrointestinal transit at the doses used in the present study.

1.5. GUIDELINES

The study procedures described were based on the guidelines listed below:

- Asetus Kokeellisiin ja muihin tieteellisiin tarkoituksiin käytettävien selkärankaisten eläinten suojelemiseksi tehdyn eurooppalaisen yleissopimuksen voimaansaattamisesta. Suomen säädöskokoelma n:o 1360/90. Helsinki, 21 joulukuuta 1990
- European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, European Treaty Series No. 123, (EU n:o 609/86) (Official Journal of the European Communities No L 358) Strasbourg 24th November 1986.

1.6. APPROVAL FROM THE ANIMAL CARE AND USE COMMITTEE

The study has a permission from the animal care and use committee of University of Turku n:o 922/99.

1.7. SPONSOR

Hormos Medical Ltd.
Tykistökatu 6A
FIN-20520 Turku
FINLAND

1.8. RESEARCH LABORATORIES

University of Turku
PreFa/Preclinical Pharmacology Research Unit
Tykistökatu 6 B
FIN-20520 Turku
FINLAND

Central Animal Laboratory
BioCity
Tykistökatu 6B
FIN-20520 Turku
Finland

CRST/Biometrics
Kiinamyllynkatu 10
FIN-20520 Turku

1.9. STUDY DIRECTOR

Aapo Honkanen, Ph.D. (Pharm.)

1.10. PERSONNEL INVOLVED IN THE STUDY

PreFa/Department of Pharmacology and Clinical Pharmacology
Esa Korpi, MD, Ph.D. Professor of Pharmacology
Aapo Honkanen, Study Director
Elisa Riuttala, Laboratory Technician

CRST/Biostastics
Esa Wallius

1.11. TIME TABLE

Start of animal acclimatisation	10.11.1999
Experimental starting date:	17.11.1999
Experimental completion date:	19.11.1999

2. MATERIALS AND METHODS

2.1. TEST SYSTEM/SUBJECTS

Experimental animals:	NMRI mice, HsdWin:NMRI
Age/weight:	7 weeks/28 ± 2 g (24-32 g)
Source:	Harlan Winkelman GmbH, Germany

Number of animals in the study:	50
Number of animals/group:	10
Acclimatisation period:	7 days before start of the experiment
Principles for selection into test groups:	Animals were randomly selected into different test groups by draw.
Identification of animals:	The animals were marked on their tails with codes in different colors
Grounds for selection of species:	Mice are commonly used in studies of this type

2.2. ENVIRONMENTAL CONDITIONS

Animal care:	The animals were cared and checked daily by the experimenters and/or personnel of the Central Animal Laboratory. The bedding of the animals were changed twice and water bottles once a week.
Number of animals/cage:	5 mice/cage.
Cage Type:	Polycarbonate Macrolon III (Scanbur AS, Denmark).
Bedding:	Aspen chips (Tapvei Oy Kaavi, Finland). The results of the analysis for specified contaminants are attached (Appendix 3).
Water:	Community tap water, *ad libitum*, except during the experiments. The results of the analysis for specified contaminants are attached (Appendix 4.).
Fodder:	RM1 (E) SQC, Special Diet Service, Witham Essex, England. Certificate detailing nutritional composition and levels of specified contaminants is attached (Appendix 5.).
Ambient temperature:	21 ± 2.5 °C
Humidity:	50 % ± 15 %
Illumination:	12-h dark/light cycle; lights on from 7.00 to 19.00 and lights of from 19.00 to 7.00.
Room numbers:	Experimental Room 314, BioCity, C-department Colony Room 309, BioCity, C-department

2.3. REAGENTS

2.3.1. Test compounds

Hydroxymatairesinol (HMR, mw. 374)

Vehicle:	PEG 300 (Sigma Chemicals Co, St Louis, MO, USA)
Batch:	00799
Storage:	at 4 °C, desiccated, protected from direct light

2.3.2. Reference compounds

Medetomidine (mw. 200.28, Domitor® 1 mg/ml,)

Manufacturer:	Orion Pharma, Espoo, Finland
Vehicle:	0.9 % NaCl (saline)
Lot:	ZH 31-3
Batch:	11/98
Storage:	at room temperature protected from direct light

2.3.3. Other reagents

5 % Charcoal (Norit "A", Pharmaceutical grade)

Vehicle:	0.8 % Methylcellulose (M0387, Sigma Chemicals Co, St Louis, MO, USA)
Manufacturer:	AMEND Drug & Chemical Co
Batch:	09/87
Storage:	at room temperature

2.3.4. Rationale for dose selection

In the experiments assessing the pharmacodynamic efficacy of HMR ,e.g. antitumor activity (Saarinen et al. Nutrition and cancer 2000 (36):207-216) a dose 15 mg/kg, (p.o.) have been found to be effective. Thus the doses selected for the present study (10, 30 and 100 mg/kg, s.c.) were within this therapeutic range or exceeded that.

2.3.5. Preparation and handling of test compound solutions

Fresh test compound solutions were prepared on each experimental day. HMR is dissolved in Polyethylene glycol (PEG) 300 and reference compound medetomidine was dissolved in 0.9% NaCl. HMR solutions were sonicated at 40 °C for 8-15 min.

2.4. EXPERIMENTS

2.4.1. Procedure

Eighteen hours before the experiment, food but not water were withdrawn and the animals were transferred into cages with a grid floor. The gastrointestinal transit (GIT) in mice was assessed by measuring the distance the charcoal meal (p.o., 0.25 ml of a 5 % suspension of charcoal) travelled from the pylorus during 15-min period following

administration of the meal. The mice were sacrificed 15 min following the charcoal meal with CO_2. The entire intestine was immediately removed, and the distance the meal had travelled through the intestine was measured

2.4.2. Administration of compounds

Vehicle or different doses of HMR were given s.c. 90 min and reference compound medetomidine 30 min before the administration of charcoal meal (Table 2.1.).

Table 2.1. Treatments

Groups	Treatment	Dose
I	Vehicle (PEG 300)	-
II	Medetomidine	50 µg/kg
VI	HMR	10 mg/kg
VII	HMR	30 mg/kg
VIII	HMR	100 mg/kg

$n_i = 10$, $n = 50$

2.4.3. Data collection

The length of small intestine from the pylorus to the caecum, and the distance of the farthest traces of the charcoal meal from the pylorus were measured and entered in the worksheet. For each animal, GIT is calculated as the percentage of distance travelled by the charcoal, relative to the total length of the small intestine.

2.4.4. Statistics

The data were tested with analysis of variance (ANOVA) and between-groups comparisons are made with Tukey post-hoc test.

2.4.5. Termination of the experiments

The animals were sacrificed with CO_2 during the experiment as described in the protocol.

3. ARCHIVING

Study plan, final report and original data from different experiments are retained in the archive of PreFa (Tykistökatu 6B) at least for 10 years. After that, the further treatment of the documentation is decided together with the Sponsor. The documentation or parts of it may be delivered to the Sponsor on request before 10-year term. No data or documentation will be destroyed without permission from the Sponsor.

4. DEVIATIONS FROM STUDY PLAN

Experiment was performed as described in the original Study Plan.

5. RESULTS

5.1. BODY WEIGHTS

Average (± S.D.) body weights of the animals in different treatment groups are shown in table 5.1. The effects of different treatments on body temperature are shown in table 5.2. There was no differences in the body weights of the animals between the groups ($F = 0.24$, $p = 0.92$, ANOVA).

Table 5.1. Average weight of the animals in each treatment group

Group	Treatment	Mean	S.D.	Min	Max	n_i
I	Veh	28	2	25	30	10
II	Medetomidine	27	1	26	30	10
VI	HMR 10	28	2	26	32	10
VII	HMR 30	28	2	24	30	10
VIII	HMR 100	28	2	25	31	10

$n_i = 10$, $n = 50$

5.2. EFFECTS ON GASTROINTESTINAL TRANSIT

ANOVA showed a significant treatment effect ($F = 25$, $p < 0.001$), which was due inhibition of transit of charcoal meal in the gut by medetomidine. Only medetomidine-treated group differed significantly from the vehicle-treated in group in the post hoc test ($p < 0.001$).

Table 5.2. Effects of HMR and medetomidine on gastrointestinal transit of charcoal meal in mice. The data is expressed as the average percentage of distance travelled by the charcoal, relative to the total length of the small intestine.

Group	Treatment	Mean	S.D.	S.E.M	Min	Max	n_i
I	Veh	42	6	2	35	52	10
II	Medetomidine	16	7	2	0	23	10
VI	HMR 10	43	8	3	34	56	10
VII	HMR 30	43	8	3	31	58	10
VIII	HMR 100	39	7	2	32	55	10

$n_i = 10$, $n = 50$

6. CONCLUSION

These results indicate that Hydroxymatairesinol does not alter gastrointestinal transit at the doses used in the present study (10-100 mg/kg, p.o.).

7. DISTRIBUTION OF THE REPORT

The Report is written in duplicate, one original copy being retained in the Archives of PreFa and one delivered to the Sponsor.

Appendices

1. Values from the individual animals
2. Statistics
3. Report from analysis of bedding for contaminants
4. Report from analysis of water for contaminants
5. Report from analysis of fodder for nutritional composition and levels of specified contaminants.